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Special regulatory T-cell review: regulation of immune responses – examining the role of T cells

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Summary

The history of regulatory T cells goes back to the realisation that T cells could provide 'help' for antibody responses: the obverse of this is their ability to hold them in check. This brief personal overview follows the initial designation of T cells as 'suppressor' and the various hypotheses, some now disproved, put forward for their mechanism of action. We now cautiously label them T regulatory cells, but realise they do not control not all immune regulation. They probably operate through several mechanisms, and some of these are discussed.

Keywords: T cells; regulation; H2 antigens; MHC; cytotoxicity; help; suppression

The regulatory role of T cells has been apparent since the discovery that they were required as helper cells for B-cell reponses. The work of Claman, Miller and Mitchison in the 1960s[1–3] laid the foundation for demonstrating the separate lineages of T and B cells, and determining the helper role of T cells for antigen-specific antibody responses. Down-regulation, or suppression of immunity, is the distaff side of help: clearly it must exist, either by cell autonomous mechanisms such as apoptosis, or by interactions of effector and/or memory cells with soluble molecules or cells to limit their function or life span.

Antigen-specific help involving cell–cell interaction was demonstrated by *in vivo* cell transfer.[3] Gershon and Kondo first raised the question of whether tolerance also involved cell interactions,[4] but when they described the transfer of suppression, provokingly naming it 'infectious tolerance', they raised a storm of protest and, indeed, had some difficulty in getting that paper published.[5]

In the early 1970s the existence of T-cell subpopulations became clear, initially from *in vivo* models of cell-mediated immunity, graft-versus-host responses,[6] extended to host-versus-graft responses against major histocompatibility complex (MHC) alloantigens *in vitro* to show the requirement for T helper cells in generating cytotoxic T-cell responses.[7–9] It was a short step to assume the existence of an additional T-cell subpopulation, and the term T suppressor cells became used as the idea of them gained ground. This was explored in a number of experimental systems, mostly in response to extrinsic antigens, although arguments for alternative mechanisms, including the now no longer talked about anti-idiotype networks, were still considered. In one instance the control of autoimmunity by a mechanism including both anti-idiotypy and suppressor T cells was proposed (see N&V commentary).[10]

By the mid-1970s it was established that MHC alloantigens came in two flavours – the determinants recognized by both the existing anti-H-2 antibodies ('SD') and allospecific cytotoxic T cells being widely expressed, while those recognized by helper T cells ('LD')[7] had limited tissue expression, but were present on B cells and macrophages (dendritic cells had not yet been separately identified).

This work also led to the realization that the H-2 'locus' in mice was a complex[11] that contained two flanking loci (H2K and H2D, encoding the 'SD' alloantigens, now called MHC class I) and an intervening stretch containing immune response ('Ir') genes controlling helper T cells for antibody responses to certain antigens.[12] 'Ia' (**I**mmune response, **a**ntigen) was the name given to this region after alloantibodies to 'LD' loci were initially described.[13] 'Ia' molecules are now called MHC class II and the molecularly identified loci are designated H2A and H2E to mirror the H2K and H2D nomenclature of MHC class I loci (but see paragraph below on cloning of the MHC). The terms 'Ia', IA and IE are anachronistic.

The discovery of MHC restriction of H2K and H2D for cytotoxic responses to viral epitopes,[14] haptens[15] and minor histocompatibility (H) antigens[16,17] was matched by the realization that helper cells were similarly MHC restricted by MHC class II molecules (then termed 'Ia').[18] The physiological role of MHC molecules and the requirement of them for T-cell responses was established.

However, this was before the cloning of the MHC region. From studies using panels of intra-H2 recombinant mouse strains it appeared that Ir genes controlling immune responses to various antigens mapped to different parts of the I region. This resulted in the original designation of IA, IB, IC, IE and IJ loci,[11] and to the attribution of genes in each subregion to the control of immunity to the relevant antigens. The IA and IE loci found favour with those working on T-cell help for B cells and for cytotoxic T-cell responses involving H2K and H2D targets,[18–20] while I-J appeared, from the work of several laboratories in Japan and the USA, to be crucial for T suppressor cells.[21]

Another crucial development in the mid-1970s was hybridomas for monoclonal antibodies.[22] That not only allowed the development of better reagents for defining MHC-encoded molecules and T-cell subpopulations, but also led to the use of hybridoma technology to make T–T hybrids.[23] Some of the first reports on these hybrids described T suppressor hybrids whose function was tested *in vitro*.[24] The numbers of hybridoma cells available allowed the performance of biochemical studies that had previously been impossible, but some of the T-cell receptor and IJ moieties reported in supernatants[25] stretched the imagination, and a certain skepticism began to grow in sections of the immunological community, further goaded by proposals from the Gershon camp of contra-suppressor T-cell circuits.[26]

What then blew the T suppressor cell story out of the water was the discovery, following cloning of the H2 complex, that the IJ region did not exist, and that the T–T suppressor hybridomas did not transcribe T-cell receptor genes.[27,28] Suddenly, T suppressor cells became the black sheep of the family, and it was a brave or foolhardy person who mentioned them by name.

However, experiments describing regulatory phenomena continued to be reported and, to avoid opprobrium, the preferred term for the cells involved has become 'regulatory T cells', or Treg cells. Many of the *in vivo* systems in which they have been described have used mice that are lymphopenic or deficient of a full T-cell reportoire;[29–31] autoimmunity is then induced in these mice. It is more difficult to produce similar results in immunosufficient mice in which the homeostatic mechanisms are intact. However, in the transplantation setting experimental transfer of tolerance to intact recipients has been reported,[32] and this type of result, together with occasional clinical reports of sustained long-term allograft survival after withdrawal of immunosuppression,[33] are consistent with the establishment of regulatory circuits.

Recently a new dimension to the story has been provided by the discovery that the transcription factor FOXP3 is expressed in Treg cells;[34] in mice at least this marker appears to be limited to T cells with regulatory function. Although in humans *FOXP3* is expressed more widely than a subpopulation of T cells, there is no doubt that humans and mice whose *FOXP3*/*Foxp3* gene is defective succumb to a disease that bears all the hallmarks of autoimmunity.[35,36]

There is currently a growth industry in ascribing all manner of T-cell regulation to this 'Treg-cell subpopulation', with the assumption of a separate lineage of $CD4^+$ $FOXP3^+$ T cells. The claims for a role of Treg cells in every ill known to mouse and humans, from cancer to autoimmunity, need critical evaluation, including investigations of the mechanism(s) of suppression. Transfer *in vivo* of suppression by a cell population of a particular phenotype is just the first step, and the effects need to be evaluated with caution. Regulatory pathways are likely to be complex and may be triggered by interactions further downstream and independent of *FOXP3* expression.

Factors other than Treg can affect the induction or expression of immune responses. To mention a few, removal of antigen by antibodies or specific cytotoxic T cells can curtail the response,[37] clonal deletion or inactivation of antigen-specific T cells can occur in the periphery as well as in the thymus,[38] mesenchymal stem cells can diminish allograft responses *in vitro* and *in vivo*, possibly by inducing cell cycle arrest,[39] transforming growth factor-β is a powerful but complex immunoregulatory mediator,[40] macrophages and dendritic cells can vary the expression of key molecules involved in calling up immunity or the obverse,[41] homeostatic control of T-cell compartments can control the expansion of one or another component, with functional effects,[42] competing clones of $CD8^+$ T cells establish immunodominant hierarchies in response to epitopes of minor H antigens in which antibodies play no part[43] and $CD8^+$ T cells can express inhibitory natural killer (NK) receptors that recognize non-classical MHC class I molecules, limiting the response to minor H determinants.[44]

The question of antigen specificity is another important aspect to address. It is relevant to understanding the mechanism(s) of regulation observed in any given situation. The long-term survival of allogeneic transplants is dependent on the continued presence of the graft, long after donor antigen-presenting cells have gone. In this case, indirect presentation by recipient dendritic cells of processed H antigens from the somatic tissue of the graft appears to drive the regulatory circuit, possibly by maintaining Treg cells and/or their mediators.[41] Since such recipients can reject third-party grafts, there must be an element of specificity in such regulation. It is important

to understand this for therapeutic manipulation because non-specific regulation would result, for example, in increased susceptibility to infection with the abrogation of graft-versus-leukaemia effects following bone marrow transplantation and donor lymphocyte infusion for treatment of leukaemias.

There is a tendency not to consider mechanisms for regulation of immunity other than 'Treg' cells. This, like attempts 'to prove a hypothesis' instead of designing experiments to disprove it, is unsound: evidence consistent with a hypothesis can be found, but it cannot be proved. It is telling, however, if experiments to disprove a hypothesis fail to do so: that strengthens the case for it.

The notion of regulatory T cells as the kingpin for T-cell-mediated regulation is a powerful one. The phenomenon of regulation clearly exists, or our lymphocyte compartments would overflow, but the mechanisms are likely to be as complex as any other in biology. They need careful unravelling rather than ascribing this function to a single cell type, otherwise the Treg cell risks going the way of the T suppressor cell.

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